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Relationship between structure and retrogradation properties of corn starch treated with 1,4-α-glucan branching enzyme

Wenwen Li [b], Caiming Li [b], Zhengbiao Gu [a, b, c, **], Yijing Qiu [b], Li Cheng [a, b], Yan Hong [a, b, c], Zhaofeng Li [a, b, c, *]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China
[b] School of Food Science and Technology, Jiangnan University, Wuxi 214122, China
[c] Synergetic Innovation Center of Food Safety and Nutrition, Jiangnan University, Wuxi, Jiangsu 214122, China

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Potassium iodide (PubChem CID: 4875)

ABSTRACT

Corn starch was selected to produce a product that undergoes slow retrogradation after enzymatic modification using the 1,4-α-glucan branching enzyme (GBE) isolated from *Geobacillus thermoglucosidans* STB02. GBE treatment of corn starch could time-dependently increase the number of α-1,6 branch points, and decrease the average chain length of amylopectin and the amylose content. Meanwhile, GBE treatment also led to reduction in setback values and relative crystallinity, indicating lower short- and long-term retrogradation of starch, respectively. After treatment for 10 h, the number of α-1,6 branch points was increased by 64.6%, while the average chain length of amylopectin and the amylose content were decreased by 11.8% and 29.5%, respectively; the setback values and relative crystallinity of starch were reduced by 45.7% and 22.0%, respectively. More importantly, the relationship between structure and retrogradation properties of corn starch treated with GBE could be fairly well described by simple linear regression. The setback value of starch was positively correlated with amylose content (R^2 = 0.9896). In starch pastes aged two weeks at 4 °C, the relative crystallinity showed positive correlations with the average chain length of amylopectin (R^2 = 0.9780) and the amylose content (R^2 = 0.9654). These results suggest that the retrogradation properties of starch can be improved by reducing the amylose content and average chain length of amylopectin, which may shed light on the further development of modified starches with low retrogradation.

1. Introduction

Starchy foods usually suffer an increase in hardness and a decline in taste during storage (Yu et al., 2013). This phenomenon is mainly caused by starch retrogradation, a process in which gelatinized starch reassociates into an ordered crystalline structure during storage (Lian, Zhu, Wen, Li, & Zhao, 2013). Many factors can affect starch retrogradation, including the structure of the starch (Ong & Blanshard, 1995), the ratio of water to starch (Mariotti, Sinelli, Catenacci, Pagani, & Lucisano, 2009), the storage conditions (Perdon, Siebenmorgen, Buescher, & Gbur, 1999), and the presence of additives (Song, Huang, Li, & Zhou, 2012). A number of physical (Kawai, Fukami, & Yamamoto, 2012; Yu et al., 2013; Zhang et al., 2014), chemical (Jensen et al., 2013), and enzymatic methods (Cai & Shi, 2010; Guraya, James, & Champagne, 2001) have been used to modify the structure of starch with the goal of reducing starch retrogradation. Among them, the enzymatic methods have the advantages of increased safety, substrate selectivity, and product specificity (Le et al., 2009).

The 1,4-α-glucan branching enzyme (GBE, EC 2.4.1.18), a member of glycosyl hydrolase family 13 (GH13), catalyzes the formation of branch points in amylose and amylopectin by cleaving an α-1,4 glucosidic bond and attaching the released linear chain, through an α-1,6 glucosidic bond, to C-6 hydroxyl position of an adjacent glucan chain (Devillers, Piper, Ballicora, & Preiss, 2003; Kajiura

* Corresponding author. School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, China.
** Corresponding author. School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, China.
E-mail addresses: zhengbiaogu@jiangnan.edu.cn (Z. Gu), zfli@jiangnan.edu.cn (Z. Li).

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et al., 2011; Takata et al., 2010). Previous reports showed that modifying starch structure using this enzyme can improve the retrogradation properties of starch. The GBE from *Streptococcus mutans* has been shown to attack rice starch, causing a large reduction in the number of longer chains and an increase in the number of branch points. As a result, starch retrogradation was reduced as the enzymatic modification of the starch increased (Kim, Ryu, Bae, Huong, & Lee, 2008). Also, amylose synergisticly modified by the GBE from *Bacillus subtilis* 168 and maltogenic amylase from *Bacillus stearothermophilus* exhibited high stability against retrogradation (Lee et al., 2008). However, little information about the relationship between structure and retrogradation properties is found.

In this study, native corn starch was treated with the GBE from *Geobacillus thermoglucosidans* STB02. The structure and retrogradation properties of corn starch treated with this GBE were investigated and the relationship between them was analyzed. These results will provide new information that may shed light on the further development of modified starches with low retrogradation.

2. Materials and methods

2.1. Materials

Native corn starch was purchased from Zhucheng Xingmao Corn Developing Co., Ltd (Shandong, China). The *gbe* gene encoding GBE was from *G. thermoglucosidans* STB02 (GenBank accession no. KJ660983), and the GBE was produced with *Escherichia coli* BL21 (DE3) harboring plasmid pET-20b (+)/*gbe*. The recombinant enzyme was purified by immobilized metal affinity chromatography using a HisTrap chelating column (5 mL, GE Healthcare). Isoamylase was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Pullulanase was purchased from Novozymes Co. (Bagsvaerd, Denmark).

2.2. Sequence analysis

The amino acid sequences of the GBEs from *G. thermoglucosidans* STB02, *S. mutans*, *B. stearothermophilus* and *B. subtilis* 168 were acquired from the NCBI (http://www.ncbi.nlm.nih.gov) database. Multiple sequence alignment was performed using CLC Sequence Viewer 6.0 software. The identity was calculated using the BLAST algorithm at the NCBI server (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Preparation of corn starch treated with GBE

Corn starch slurry (30%, w/v) was prepared in deionized water, and adjusted to pH 7.5. The slurry was incubated with GBE (300 U/g starch) in a water bath at 50 °C for 10 h. Then, the starch sample was washed with 10 volumes of deionized water and dried in a vacuum desiccator. After passing through a 100-mesh sieve, the corn starch treated with GBE was obtained. The control was the corn starch treated without GBE.

2.4. Determination of glycosidic linkage ratio

The glycosidic linkage ratio of corn starch treated with GBE was determined using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy (Avance III-400 MHz, Bruker Co. Germany). Samples of corn starch treated with GBE were dissolved in deuterium oxide (D_2O) to a final concentration of 80 mg/mL (w/v) and then boiled with stirring for 30 min. These samples were then freeze-dried and redissolved in D_2O to a concentration of 80 mg/mL (w/v). The ^{1}H NMR spectra of these samples were collected at 60 °C (Lee et al., 2013). The α-1,6 glycosidic linkage ratio was calculated by dividing the area of α-1,6 glycosidic linkage peak by the total area of α-1,4 glycosidic linkage and α-1,6 glycosidic linkage peaks in the NMR spectra.

2.5. Determination of debranched linear chain length distribution

The linear chain length distribution of debranched starch was determined using high-performance anion-exchange chromatography (HPAEC) as follows. A portion of each starch sample was suspended in sodium acetate buffer (50 mM, pH 4.0) to a final concentration of 0.5% (w/v). This slurry was gelatinized in a boiling water bath for 30 min while stirring. After cooling to room temperature, the mixture was incubated with isoamylase (10,000 U/g substrate) and pullulanase (6000 U/g substrate) at 40 °C for 24 h to ensure complete debranching. The debranching reaction was stopped by boiling for 10 min. After centrifugation (8000 r/min for 20 min) and filtration (0.45 μm) to clarify sample, it was analyzed using an HPAEC system (ICS-5000, Dionex Co., USA) equipped with a pulsed amperometric detector (PAD) and a CarboPac PA200 column (3 × 200 mm) (Roussel et al., 2013). The chain length distribution was characterized as a percentage of the total peak area.

2.6. Determination of amylose content

The amylose content was determined by measuring the absorbance at 720 nm resulting from reaction of starch samples with an iodine solution (Biselli et al., 2014; Jiang et al., 2015). A sample (0.1 g) was combined with 1 mL ethanol (95%, v/v) and 9 mL NaOH solution (1 M), and then heated in a boiling water bath for 10 min. After cooling, the sample was transferred to a volumetric flask and diluted with deionized water to 100 mL. A 5 mL aliquot of the mixture was pipetted into a 100-mL volumetric flask and combined with 1 mL acetic acid (1 M) and 2 mL I_2 solution (0.02% (w/v) KI, 0.002% (w/v) I_2). The mixture was diluted to 100 mL with deionized water, shaken, and then allowed to stand for 10 min. The absorbance of the final solution at 720 nm was converted to the amylose content using a standard curve.

2.7. Pasting properties

The pasting properties of starch samples at a concentration (dry basis) of 6% (w/v) were determined using a Brabender viscograph (Brabender OHG, Germany). The temperature-time conditions for each measurement were as follows. The sample was heated from 35 to 95 °C at 1.5 °C/min, held at 95 °C for 30 min, cooled from 95 to 50 °C at 1.5 °C/min, and then held at 50 °C for 30 min. The rotation speed was 75 rpm (Tang, Hong, Gu, Zhang, & Cai, 2013).

2.8. X-ray diffraction analysis

Each starch sample was suspended in deionized water (25 g) to a final concentration of 6% (w/w). The mixture was pasted using a rapid viscosity analyzer (RVA-Tecmaster, Newport Scientific Pty. Ltd., Australia). After cooling to room temperature, the sample was stored at 4 °C for 14 days, and then freeze-dried. The freeze-dried sample was passed through a 100-mesh sieve. Then the powders were placed in a sealed glass desiccator containing saturated sodium chloride (75% relative humidity) to achieve a uniform moisture content of around 9% (Sievert, Czuchajowska, & Pomeranz, 1991). Wide-angle X-ray scattering measurements of these freeze-dried samples were performed at 40 kV using 30 mA Cu-Kα radiation in an X-ray diffractometer (Bruker AXS Inc., Germany). Diffractograms were obtained by scanning from 5° to 35° (2θ) at a rate of 2°/min and a step size of 0.02°. The divergence slit width was